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(54) Abstract Title
Antioxidant peptides

(57) An antioxidant compound is disclosed. The compound is characterized by (a) a peptide including at least three amino acid residues of which at least one being a cysteine residue having a readily oxidizable sulfhydryl group for effecting antioxidation; and at least one peptide bond cleavable by at least one intracellular peptidase; and (b) a first hydrophobic or non-charged moiety being attached to an amino terminal of the peptide via a first bond and a second hydrophobic or non-charged moiety being attached to a carboxy terminal of the peptide via a second bond, the first hydrophobic or non-charged moiety and the second hydrophobic or non-charged moiety are selected so as to provide the antioxidant compound with membrane miscibility properties for permitting the antioxidant compound to cross cellular membranes; wherein cleavage of the at least one peptide bond by the at least one intracellular peptidase results in generation of at least one antioxidant species including the cysteine residue having the readily oxidizable sulfhydryl group and which is also active in effecting antioxidation, thereby providing for a plurality of different antioxidant species The compounds may be used in pharmaceutical composition for reducing oxidative stress, and are effective at crossing blood barriers, such as the blood brain barrier, blood retinal barrier or blood testis barrier.

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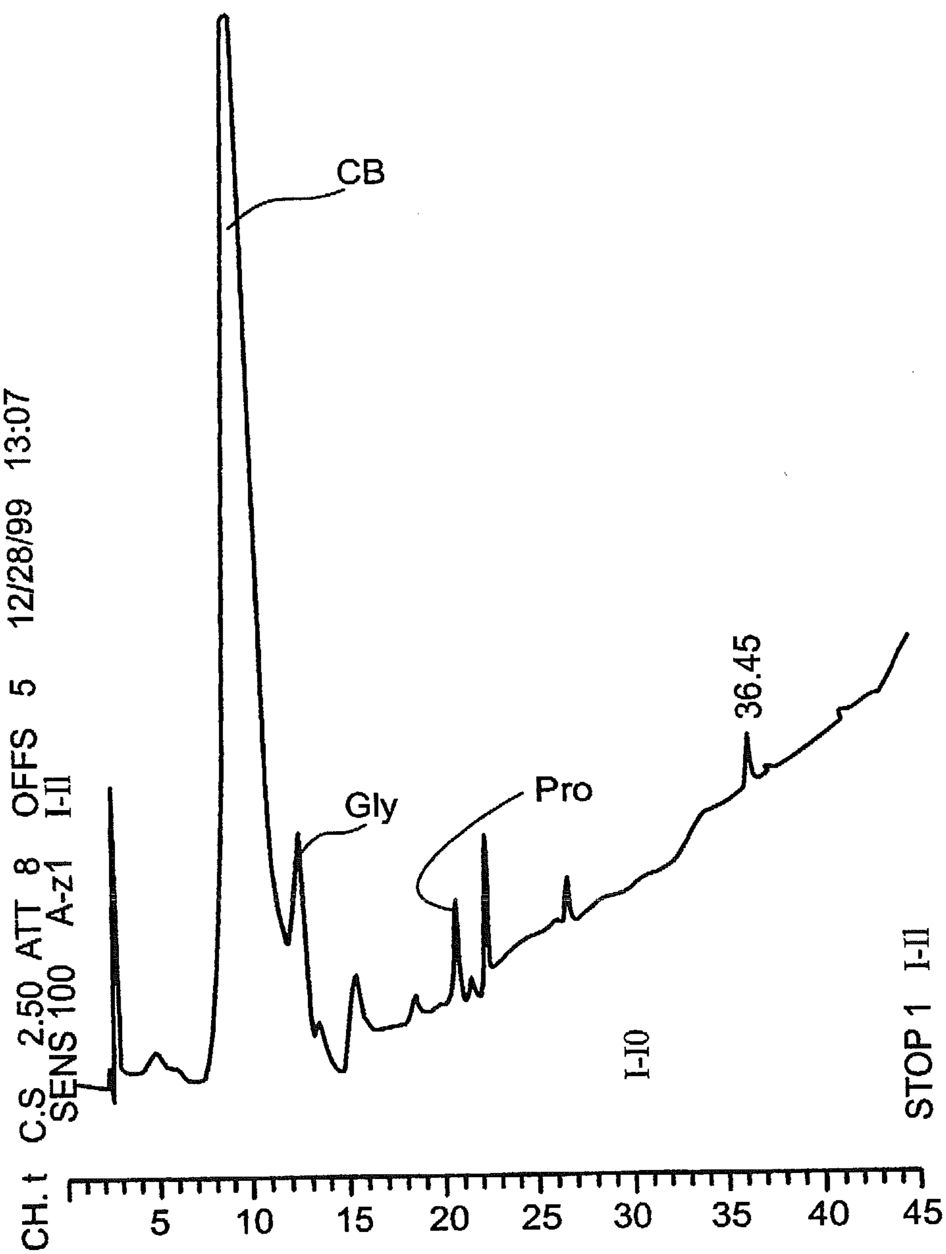
At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

Solid Phase Synthesis of CB



CB-retention time at 9.62 min

Fig. 1

Protection from Reactive Oxygen Species (ROS)
by CB and NOXi

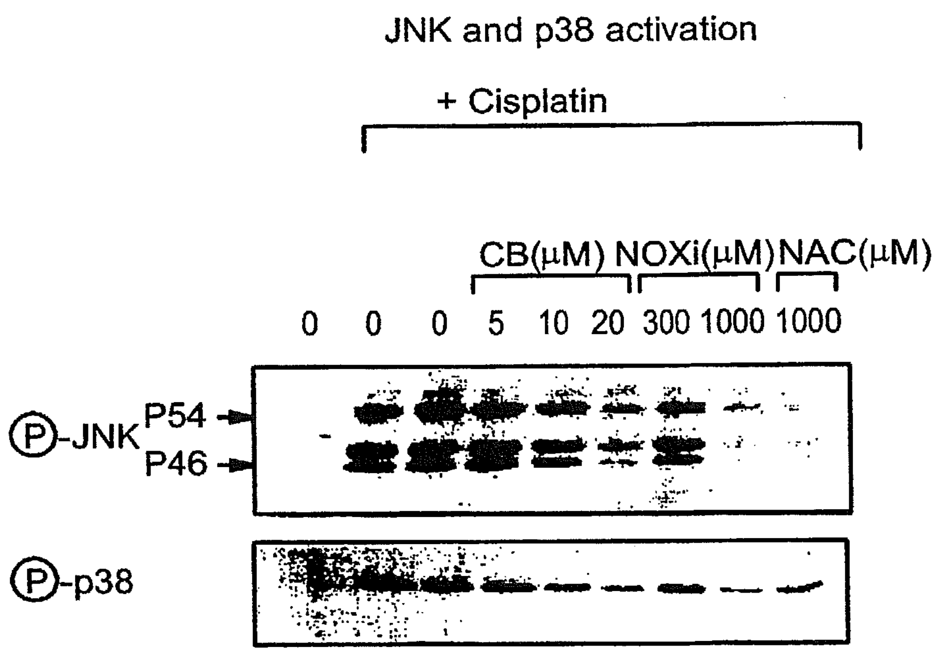


Fig. 2

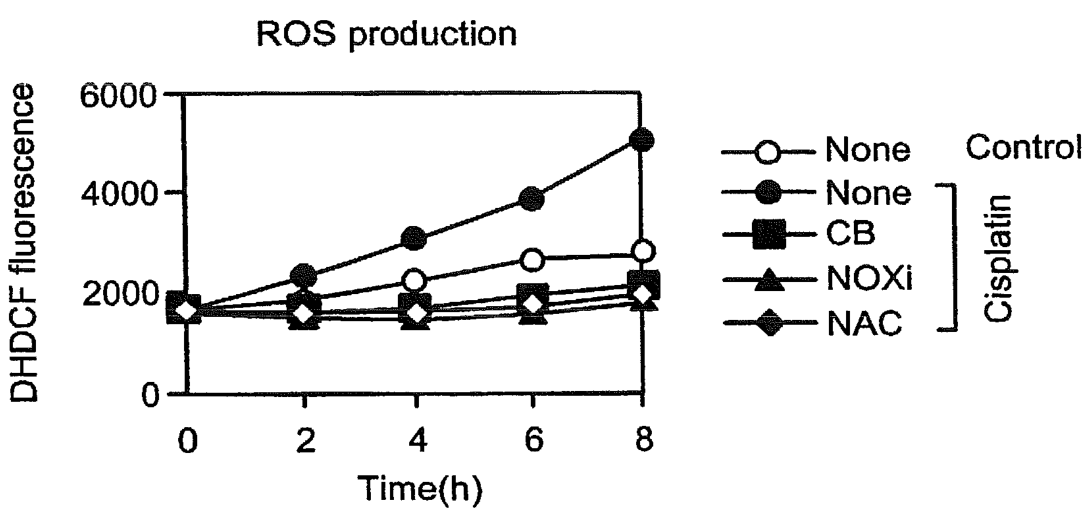


Fig. 3

APPLICATION FOR PATENT

Inventor: Daphne Atlas

Title: MULTI-COMPONENT ANTIOXIDANT COMPOUNDS, PHARMACEUTICAL COMPOSITIONS CONTAINING SAME AND THEIR USE FOR REDUCING OR PREVENTING OXIDATIVE STRESS

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to antioxidant compounds, pharmaceutical compositions containing same and their use for preventing or reducing oxidative stress. More particularly, the present invention relates to novel peripheral and brain targeted antioxidants and their use in treating peripheral and brain disorders, diseases or conditions associated with a formation of oxidative stress.

Oxidation –Reduction processes inside a cell are balanced by specific enzymes such as superoxide dismutase, catalase, gluthation peroxidase and thioredoxin, and selective antioxidants such as glutathione. Impairment of the delicate redox balance by an excess of reactive oxygen species generates an oxidative state which is called oxidative stress and which has been implicated in many diseases ranging from atherosclerosis to ischemic-reperfusion injury to cancer.

Recently, several neurodegenerative disorders were characterized by an over production of reactive oxygen species, which exceeds the capacity of the cellular antioxidant defenses to neutralize them. This situation leads

to oxidant injury. Treatment of such conditions is strategically directed either toward prevention of enzymatic production of reactive oxygen species (ROS) by specific inhibitors or by introduction of exogenous antioxidant compounds that restore the reduction- oxidation (redox) balance in biological systems.

Correlation between oxidative stress and cellular processes

Accumulating evidence shows that alterations in the cellular oxidation-reduction (redox) state play a critical role in cell signaling through modulation of tyrosine phosphorylation, regulation of transcription and alterations in messenger RNA stability. Moreover, reactive oxygen species (ROS) are thought to participate in many cellular processes including differentiation, proliferation, and apoptosis (programmed cell death). Addition of ROS or depletion of cellular antioxidants causes apoptosis (Buttke and Sandstorm, 1994) and many cytotoxic pathways are accompanied by an increase in the total level of ROS (Jacobson, 1996). In dealing with such alterations in the cellular oxidation-reduction (redox) state, it was suggested that administration of antioxidants or sulfhydryl-reactive agents may have important modulatory effects on cellular processes. Furthermore, previously, it was demonstrated that addition of antioxidants such as N-acetyl cysteine displayed significant protection against ROS-mediated cell death induced by TNF-α (Mayer and Nobel, 1994; Cossarizza et al., 1995). Current studies have shown that management of both cancer and AIDS by redox-sensitive mediated pathways can be added to the steadily growing list of redox-sensitive signal transduction pathways.

The development and maintenance of a healthy tissue involves programmed cell death (apoptosis). Interference with this process contributes to various pathologies including tumor promotion, immunodeficienciy diseases and neuerodegenerative disorders. In recent years, much evidence have accumulated linking oxidative stress, UV

radiation and cisplatinum treatment to activating specific enzymes within cells in the apoptosis process. One of the essential enzymes in the signaling pathway of apoptosis is c-Jun, an NH_2-terminal kinase (JNK) which is activated in response to UV radiation, cisplatinum treatment or cytotoxic stress. Recently it was demonstrated that disrupting the JNK genes protected against UV induced apoptosis, resulting in impairment of mitochondria death signaling pathway (Tournier et al., 2000).

In a study by Saitoh et al., (1998), ROS were shown to play a role as intermediate factors in the pathway of various signal transduction involving thioredoxin, a ubiquitous enzyme in all living cells, characterized by a specific reduction-oxidation (redox) active site. Due to its redox active-center thioredoxin acts as an inhibitor of Apoptosis Signal regulating Kinase (ASK1), a protein that mediates stress induced apoptosis. It was shown that interaction between thioredoxin and ASK1 was affected by ROS and once thioredoxin was oxidized, ASK1 was activated, which led to apoptosis. Hence, thioredoxin acts as a negative inhibitor of stress mediated apoptosis. Whenever thioredoxin is oxidized by ROS, it dissociates from ASK1 thus activating it, allowing the cells to proceed toward apoptosis. Hence, a large excess of ROS is linked directly to cell death. Use of antioxidants which provide additional reducing power to the cells in peripheral tissue, could rescue the above cells from an early apoptosis.

Correlation between oxidative stress inflammation and various neurodegenerative pathologies

In the last few years accumulated evidences have demonstrated a strong linkage of oxidative stress with pathogenesis such as Parkinson's, Alzheimer's Creutzfeldt-Jakob's diseases and other human neurodegenerative disorders (Olanow, 1990, 1993; Fahn and Cohen, 1992; Jones et al., 2000, Brown *et al.*, 1996; Thomas *et al.*, 1996; Nunomura et al., 1999) as well as various processes of inflammation such as multiple sclerosis (MS) (Mann et al., such as elevation of the pro-inflammatory

cytokines one of which is TNF-alpha (tumor-necrosis factor alpha). During the inflammatory process TNF-alpha interacts with its receptor followed by the activation of a cascade of reactions, as a result free radicals are generated in the cell. These free radicals are an intermediate step in the activation of selective stress pathways that lead to cell death. Indeed, in all inflammatory associated diseases such as sepsis, multiple sclerosis, stroke, myocarditis, rheumatoid arthritis, etc., an excess of free radicals had been identified. Treatment with antioxidants, e.g., N-acetyl cysteine (NAC) or GSH was shown to be effective at protecting the cells from apoptosis.

The different pathological makers of various neurodegenerative diseases e.g., Lewy bodies, plaques, etc., indicate different causal factors in the initiation of these diseases. However, there is growing evidence that once initiated, the progression of a large number of neurodegenerative diseases, resumes similar cellular pathways. Although the characteristic symptoms are descriptive for each neurodegenerative disease, it appears that elevation of the oxidative state (OS) of the cells at specific regions in the brain is an important factor in the etiology of Parkinson's disease, basal ganglia degenerative diseases, motoneuron diseases, Alzheimer's, Creutzfeldt-Jakob's diseases, loss or impaired memory and other syndromes and diseases.

An indication for a role played by oxidative stress in the pathogenesis of Alzheimer's disease was found while in a recent study, the relationship between the β-amyloid protein fragments and oxygen radical formation was tested in a system that is highly sensitive and responds to free oxygen radicals. This system utilizes the vasoactivity of the blood vessel, which in the presence of β-amyloid, enhances phenylephrine mediated-contraction of the vessels. Pretreatment of the blood vessel with superoxide dismutase (SOD), an enzyme which scavenges free oxygen radicals, eliminated the effect of β-amyloid, namely, there was no enhancement of vasoconstriction. Whereas, when SOD was added following treatment with β-amyloid

protein, the protective effect of the radical scavenger was abolished (Thomas *et al.*, 1996). Other studies have shown that oxidative stress and free radicals production are linked to β-amyloid fragment which includes amino acids 25-35 and may contribute to neurodegenerative events associated with Alzheimer's disease (Cafe *et al.*, 1996).

More recent studies show that RNA oxidation is a prominent feature of neurons in Alzheimer's disease (Nunomura et al., 1999a,b) and genetic evidence for oxidative stress in Alzheimer's disease was recently reported (Raina et al., 1999; see also the review by Rottkamp et al., 2000)

Possible indications for the role of oxidative stress in the pathogenesis of Scrapie, spongyform encephalopathy (BSE) and Creutzfeldt-Jakob's diseases are listed hereinbelow.

Already in 1996, it was demonstrated that the toxic effect of Scrapie requires the presence of microglia cells, which respond to a prion protein fragment (PrP106-126) by increasing their oxygen radical production. Interestingly, all these effects were dependent on mice that express the prion protein PrP^C (Brown *et al.*, 1996). The contribution of progressive oxidative stress to the state of various diseases and to the mechanism of cell death is further demonstrated in a study by P. Jenner in The Lancet (1994) 344, 796-798, which is incorporated by reference as if fully set forth herein.

New therapeutic aspects for preventing or reducing oxidative stress

The use of glial cell-derived neurotrophic factor (GDNF) was established as a potential stimulant for the increase of dopamine levels in midbrain of rhesus monkeys for treatment of Parkinson's disease (Gash *et al.*, 1996). This study which extends previous results obtained with rodents, is promising as to the provision of a potential treatment for Parkinson's disease. However, like any other protein, GDNF cannot cross the blood brain barrier. Therefore, it can not be taken orally or be injected systemically. The only possible mode of administration would thus be via

an intracerebral injection and would constitute a major drawback for such a treatment.

Similarly, in other neurodegenerative diseases such as Alzheimer's and Creutzfeldt-Jakob's, where the theory of free oxygen radicals appears to play a major role, there is no major breakthrough in therapy.

To overcome high oxidative stress for the treatment of diseases of the brain it would be beneficial to augment the reduced state of the cells at the central nervous system (CNS). Recently, a similar approach for reducing the levels of free oxygen, was taken for the treatment of asthma (Bundy *et al.*, 1995). A reactive oxygen inhibitor was synthesized (2,4-diaminopyrrolo-[2,3-dipyrimidines) and after a successful pharmacological bio-availability and toxicity tests was selected for clinical evaluation. This approach for the reduction of OS in peripheral tissues would be applicable for the brain.

One of the possible ways to do so is by increasing the level of reduced glutathione, increase in the level of reduced thioredoxin or other scavengers of free radicals and free oxygen in the brain.

In general, in order to lower oxidative stress levels, various antioxidants are being used. The most common are vitamin E and vitamin C. However, vitamin E was found to be ineffective at decreasing the oxidative stress at the substantia nigra (The Parkinson Study Group, 1993, Offen *et al.*, 1996) since this compound, although capable of crossing the blood brain barrier, is trapped in the cell membrane and therefore does not reach the cytoplasm where its antioxidant properties are needed. Vitamin C was shown to cross the blood brain barrier to some extent via a selective transporter, nevertheless it was shown to be rather effective for treating neurodegenerative diseases of central origin.

A somewhat different approach involves stimulating the production of endogenous antioxidants, especially reduced glutathione. To this end a drug known as Pro-cysteine which boosts cellular production of glutathione

by loading the cells with cysteine is under clinical trials these days by Free Radical Sciences Inc. (CA, U.S.) to treat conditions of acute respiratory distress syndrome (ARDS) which includes overproduction of oxidants or reactive oxygen species by the immune system. Other conditions in which overproduction of oxidants is experienced include but are not limited to amyotrophic lateral sclerosis, atherosclerotic cardiovascular disease and multiple organ dysfunction. See, for example, Charles Craig, 1996.

U.S. Pat. No. 5,874,468 and PCT/US97/23997 describe antioxidant compounds characterized by (a) a combination of low molecular weight and membrane miscibility properties for permitting the compounds to cross the blood brain barrier of an organism; (b) a readily oxydizable (i.e., reducing) chemical group for exerting antioxidation properties; and (c) a chemical make-up for permitting the compounds or their intracellular derivative to accumulate within the cytoplasm of cells. Specific examples include N-acetyl cysteine ethyl ester, β,β-dimethyl cysteine ethyl ester, N-acetyl-β,β-dimethyl cysteine, glutathione ethyl ester, N-acetyl glutathione ethyl ester, N-acetyl glutathione, N-acetyl (α ethyl ester) glutathione N-acetyl (α ethyl ester) glutathione ethyl ester, N-acetyl glutathione amide, N-acetyl cysteine amide, N-acetyl β,β dimethyl cysteine amide and N-acetyl cysteine glycine amide.

A common feature characterizing all of the above described and other antioxidant compounds is their limited diversity in structure, body distribution, cellular distribution, organelle distribution, and/or antioxidant properties, etc. As such, any given antioxidant may prove useful for some applications, yet less or non-useful for other applications. In some cases, a specific antioxidant may efficiently reduce oxidative stress in some body parts, some cells, or some subcellular structures, yet not in other.

There is thus, a great need for, and it would be highly advantageous to have an antioxidant compound which is devoid of the above limitations, which compound will by hydrolyzed *in vivo* to a plurality of different

antioxidant species which will act in concert to reduce or prevent oxidative stress in a plurality of tissues, cell types and cellular organelles, so as to combat disease, syndromes and conditions associated with formation of oxidative stress both in the body periphery and in the brain.

SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided an antioxidant compound comprising (a) a peptide including at least three amino acid residues of which at least one being a cysteine residue having a readily oxidizable sulfhydryl group for effecting antioxidation; and at least one peptide bond cleavable by at least one intracellular peptidase; and (b) a first hydrophobic or non-charged moiety being attached to an amino terminal of the peptide via a first bond and a second hydrophobic or non-charged moiety being attached to a carboxy terminal of the peptide via a second bond, the first hydrophobic or non-charged moiety and the second hydrophobic or non-charged moiety are selected so as to provide the antioxidant compound with membrane miscibility properties for permitting the antioxidant compound to cross cellular membranes; wherein cleavage of the at least one peptide bond by the at least one intracellular peptidase results in generation of at least one antioxidant species including the cysteine residue having the readily oxidizable sulfhydryl group and which is also active in effecting antioxidation, thereby providing for a plurality of different antioxidant species.

According to another aspect of the present invention there is provided a pharmaceutical composition for preventing or reducing oxidative stress, the composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, an effective amount of an antioxidant compound, the antioxidant compound including: (a) a peptide including at

least three amino acid residues of which at least one being a cysteine residue having a readily oxidizable sulfhydryl group for effecting antioxidation; and at least one peptide bond cleavable by at least one intracellular peptidase; and (b) a first hydrophobic or non-charged moiety being attached to an amino terminal of the peptide via a first bond and a second hydrophobic or non-charged moiety being attached to a carboxy terminal of the peptide via a second bond, the first hydrophobic or non-charged moiety and the second hydrophobic or non-charged moiety are selected so as to provide the antioxidant compound with membrane miscibility properties for permitting the antioxidant compound to cross cellular membranes; wherein cleavage of the at least one peptide bond by the at least one intracellular peptidase results in generation of at least one antioxidant species including the Cysteine residue having the readily oxidizable sulfhydryl group and which is also active in effecting antioxidation, thereby providing for a plurality of different antioxidant species.

According to yet another aspect of the present invention there is provided a method of preventing or reducing oxidative stress, the method comprising the step of administering to a subject an effective amount of an antioxidant compound, the antioxidant compound including: (a) a peptide including at least three amino acid residues of which at least one being a cysteine residue having a readily oxidizable sulfhydryl group for effecting antioxidation; and at least one peptide bond cleavable by at least one intracellular peptidase; and (b) a first hydrophobic or non-charged moiety being attached to an amino terminal of the peptide via a first bond and a second hydrophobic or non-charged moiety ~~being~~ attached to a carboxy terminal of the peptide via a second bond, the first hydrophobic or non-charged moiety and the second hydrophobic or non-charged moiety are selected so as to provide the antioxidant compound with membrane miscibility properties for permitting the antioxidant compound to cross cellular membranes; wherein cleavage of the at least one peptide bond by

the at least one intracellular peptidase results in generation of at least one antioxidant species including the cysteine residue having the readily oxidizable sulfhydryl group and which is also active in effecting antioxidation, thereby providing for a plurality of different antioxidant species.

According to further features in preferred embodiments of the invention described below, the antioxidant compound has a general formula of:

A---Y1---Cys---Y2---Cys---Y3---B

or

A---Y1---Cys---Y2---B

wherein, Cys is a cysteine residue, A is the first hydrophobic or non-charged moiety; B is the second hydrophobic or non-charged moiety; Y1, Y2 and Y3 are each individually one or more amino acid residues in the range of 0-30 residues, with the provision that Y1, Y2 and Y3 collectively provide for at least two amino acid residues in the peptide.

According to still further features in the described preferred embodiments the A is selected from the group consisting of N-acetyl, tert butyl, iso propyl, n-butyl and n-pentyl. According to still further features in the described preferred embodiments the B is selected from the group consisting of amide and ester.

According to still further features in the described preferred embodiments cleavage of the first bond and/or the second bond by a cellular hydrolase results in loosing the membrane miscibility.

According to still further features in the described preferred embodiments the cleavage of the first bond and/or the second bond by a cellular hydrolase results in formation of additional antioxidant species acting in synergy.

According to still further features in the described preferred embodiments the first bond and the second bond are each independently an ester or peptide bond.

According to still further features in the described preferred embodiments each of the first hydrophobic or non-charged moiety and the second hydrophobic or non-charged moiety is selected from the group consisting of alkyl, aryl, alkene, arene and cholesteril having a backbone of 2-50 carbon atoms.

According to still further features in the described preferred embodiments the first hydrophobic or non-charged moiety and the second hydrophobic or non-charged moiety are selected so as to enable the antioxidant compound to cross a blood barrier.

According to still further features in the described preferred embodiments the blood barrier is selected from the group consisting of a blood brain barrier, a blood retinal barrier and a blood testis barrier.

According to still further features in the described preferred embodiments the plurality of different antioxidant species act in synergy to exert antioxidation.

According to still further features in the described preferred embodiments the pharmaceutically acceptable carrier is selected from the group consisting of a thickener, a base, a buffer, a diluent, a surface active agent and a preservatives.

According to still further features in the described preferred embodiments the subject is a human being.

According to still further features in the described preferred embodiments the subject has a peripheral disorder characterized by formation of oxidative stress.

According to still further features in the described preferred embodiments the peripheral disorder characterized by formation of oxidative stress is selected from the group consisting of an acute respiratory

distress syndrome, an amyotrophic lateral sclerosis, an atherosclerotic cardiovascular disease, multiple organ dysfunction, complication resulting from inflammatory processes, AIDS, cancer and aging.

According to still further features in the described preferred embodiments the subject has a neurodegenerative disorder characterized by oxidative stress.

According to still further features in the described preferred embodiments the neurodegenerative disorder characterized by oxidative stress. is selected from the group consisting of Parkinson's disease, Alzheimer's disease, Creutzfeldt-Jakob's disease, cerebral ischemia, Multiple Sclerosis, basal ganglia degenerative disease, motoneuron diseases, Scrapie, spongyform encephalopathy and loss or impaired memory.

According to still further features in the described preferred embodiments the subject has at least one habit resulting in oxidative stress.

According to still further features in the described preferred embodiments the habit is selected from the group consisting of smoking, sun tanning, cancer treatment, radiation cocaine consumption and morphine consumption.

According to still further features in the described preferred embodiments the administration is a peripheral administration.

According to still further features in the described preferred embodiments the peripheral administration is selected from the group consisting of topical administration, oral administration, administration by inhalation, and parenteral administration.

The present invention successfully addresses the shortcomings of the presently known configurations by providing a of novel multifunctional antioxidant compounds which are peripheral and brain targeted antioxidants, N- and/or C- terminal blocked peptide derivatives for the use in treatment of peripheral and brain disorders related to oxidation processes.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIG. 1 shows the HPLC profile of purified N-Acetyl Cysteine-Glycine-Proline-Cysteine-Amid (referred to herein as CB, SEQ ID NO:1)) compound according to the present invention;

FIG. 2 shows inhibition of JNK and p38 phosphorylation by CB, NOXi and NAC as determined by immunoprecipitation with specific antibodies against phosphorylated JNK and p38 followed by gel electrophoresis;

FIG. 3 represents cellular ROS levels as determined using a fluorescence assay in the presence of CB, NOXi and NAC antioxidants.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of novel peripheral and brain targeted antioxidant compounds and their use in treating peripheral and brain disorders, diseases or conditions associated with the formation of oxidative stress. More specifically, the compounds of the present invention can be

used for treatment of neurodegenerative disorders in which the pathology in the brain is associated with oxidative stress, and for treatment of peripheral tissue in conditions associated with overproduction of oxidants. Moreover, the novel compounds of the present invention can also be used for improving cognitive skills such as memory.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or illustrated in the examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

The principles of operation of the compounds according to the present invention may be better understood with reference to the examples and accompanying descriptions.

Antioxidant compounds are used according to the present invention to relieve oxidation stress within cells. A compound which is used to relieve oxidation stress according to the present invention (i) has a combination of molecular weight and membrane miscibility properties rendering it capable of crossing blood barriers; (ii) includes a readily oxidizable (i.e., reduced) chemical group, such as, but not limited to, a sulfhydryl (-SH) group derived from a cysteine amino acid residue, for exerting its antioxidation properties; and (iii) has a chemical make-up for permitting it or its cellular derivative(s) to accumulate within the cytoplasm of cells. Collectively, these properties render the compounds of the present invention suitable for treatment of neurodegenerative disorder of the central nervous system, as well as for treating conditions in which peripheral tissues, such as, but not limited to, the lungs and/or heart, are damaged due to overproduction of oxidants (i.e., reactive oxygen species), which is the case in, for example, acute respiratory distress syndrome, amyotrophic

lateral sclerosis, atherosclerotic cardiovascular disease, multiple organ dysfunction, complication resulting from inflammatory processes, AIDS, cancer and aging.

As is further detailed in the background section above, prior art antioxidant compounds are limited in their structure diversity, body distribution, cellular distribution, organelle distribution, and/or antioxidant properties and capabilities, etc. As such, prior art antioxidant compounds are useful for some applications, yet less or non-useful for other applications.

To overcome the limitations inherent to prior art antioxidant compounds and their use, the present invention teaches novel compounds which are hydrolyzed in vivo to a plurality of different antioxidant species, which act in concert to reduce or prevent oxidative stress in a plurality of tissues, cell types and cellular organelles, so as to combat disease, syndromes and conditions associated with formation of oxidative stress both in the body periphery and in the brain.

Thus, according to one aspect of the present invention there is provided an antioxidant compound which includes a peptide including at least three amino acid residues of which at least one is a cysteine residue having a readily oxidizable sulfhydryl group which serves for effecting antioxidation. The peptide, which is an antioxidant in itself, also includes at least one peptide bond cleavable by at least one intracellular peptidase. The antioxidant compound of the present invention further includes a first hydrophobic or non-charged moiety which is attached to an amino terminal of the peptide via a first bond and a second hydrophobic or non-charged moiety which is attached to a carboxy terminal of the peptide via a second bond. The first and second hydrophobic or non-charged moieties are selected so as to provide the antioxidant compound with membrane miscibility properties, for permitting the antioxidant compound to cross cellular membranes. The antioxidant compounds of the present invention

are characterized by the following unique and advantageous feature. Cleavage of the peptide bond(s) of the peptide by the intracellular peptidase(s) results in generation of at least one antioxidant species including the cysteine residue and having the readily oxidizable sulfhydryl group and which is also active in effecting antioxidation, thereby providing a plurality of different antioxidant species.

Thus, the antioxidant compound of the present invention is a peptide prodrug which penetrates the cells due to its solubility in the cell membrane. Upon entering the cytoplasm of a cell, the prodrug is cleaved by one or several intracellular peptidases, to release a plurality of different antioxidant species, each having at least one readily oxidizable sulfhydryl group to exert the antioxidative properties and acting in synergy. Each cleaved species acts according to its biological half-life and independently of the other generated species to exert antioxidation. It will be appreciated in this respect that different cells consist of a selective set of different peptidases/esterases.

As used herein in the specification, the term "prodrug" refers to an agent which is converted into an active parent drug *in vivo*. Prodrugs are often useful because in some instances they may be easier to administer than the parent drug itself. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility compared to the parent drug in pharmaceutical compositions.

As used herein in the specification and in the claims section below the term "peptide" includes native peptides (either degradation products, synthetically synthesized peptides or recombinant peptides) and peptidomimetics (typically, synthetically synthesized peptides), such as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body, or less immunogenic. Such modifications include, but are not limited to, cyclization, N-terminus modification, C-terminus modification, peptide

bond modification, including, but not limited to, CH_2-NH, CH_2-S, CH_2-S=O, O=C-NH, CH_2-O, CH_2-CH_2, S=C-NH, CH=CH or CF=CH, backbone modification and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further detail in this respect are provided hereinunder.

Thus, a peptide according to the present invention can be a cyclic peptide. Cyclization can be obtained, for example, through amide bond formation, e.g., by incorporating Glu, Asp, Lys, Orn, di-amino butyric (Dab) acid, di-aminopropionic (Dap) acid at various positions in the chain (-CO-NH or -NH-CO bonds). Backbone to backbone cyclization can also be obtained through incorporation of modified amino acids of the formulas H-N((CH_2)$_n$-COOH)-C(R)H-COOH or H-N((CH_2)$_n$-COOH)-C(R)H-NH_2, wherein n = 1-4, and further wherein R is any natural or non-natural side chain of an amino acid.

Cyclization via formation of S-S bonds through incorporation of two Cys residues is also possible. Additional side-chain to side chain cyclization can be obtained via formation of an interaction bond of the formula -(-CH_2-)$_n$-S-CH_2-C-, wherein n = 1 or 2, which is possible, for example, through incorporation of Cys or homoCys and reaction of its free SH group with, e.g., bromoacetylated Lys, Orn, Dab or Dap.

Peptide bonds (-CO-NH-) within the peptide may be substituted, for example, by N-methylated bonds (-N(CH_3)-CO-), ester bonds (-C(R)H-C-O-O-C(R)-N-), ketomethylen bonds (-CO-CH_2-), α-aza bonds (-NH-N(R)-CO-), wherein R is any alkyl, e.g., methyl, carba bonds (-CH_2-NH-), hydroxyethylene bonds (-CH(OH)-CH_2-), thioamide bonds (-CS-NH-), olefinic double bonds (-CH=CH-), retro amide bonds (-NH-CO-), peptide

derivatives (-N(R)-CH_2-CO-), wherein R is the "normal" side chain, naturally presented on the carbon atom.

These modifications can occur at any of the bonds along the peptide chain and even at several (2-3) at the same time.

Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted for synthetic non-natural acid such as TIC, naphthyl (Nol), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr.

Accordingly, as used herein in the specification and in the claims section below the term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally *in vivo*, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids which are linked via a peptide bond or a peptide bond analog to at least one addition amino acid as this term is defined herein.

An amino acid residue is understood to be an amino acid as this term is defined herein when serving as a building block or unit in a peptide, as this term is defined herein.

Tables 1-2 below list all the naturally occurring amino acids (Table 1) and non-conventional or modified amino acids (Table 2).

TABLE 1

Amino Acid	Three-Letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F

Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any amino acid as above	Xaa	X

TABLE 2

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgin
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcyclopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α- methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N- amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex

D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cyclododeclglycine	Ncdod
D-α-methylalnine	Dnmala	N-cyclooctylglycine	Ncoct
D-α-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-α-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-α-methylasparatate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-α-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl) glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nva
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomo phenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn

L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	mser	L-α-methylthreonine	Mthr
L-α-methylvaline	Mtrp	L-α-methyltyrosine	Mtyr
L-α-methylleucine	Mval Nnbhm	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl-glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl(1)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl ethylamino)cyclopropane	Nmbc		

According to a presently preferred embodiment of the invention, the antioxidant compound has the general formula:

A---Y1---Cys---Y2---Cys---Y3---B

or

A---Y1---Cys---Y2---B

wherein, Cys is a cysteine residue, A is the first hydrophobic or non-charged moiety; B is the second hydrophobic or non-charged moiety; Y1, Y2 and Y3 are each individually one or more amino acid residues in the range of 0-30, preferably 0-20, more preferably 0-10, most preferably 0-5, 0-4, 0-3, 0-2 or 0-1 amino acid residues, with the provision that Y1, Y2 and Y3 collectively provide for at least two amino acid residues in the peptide.

A compound which has the above listed properties and which is hydrolyzable within a cell so as to generate a plurality of antioxidant species acting in concert is for example:

A-Cys-A1-A2-Cys-B (SEQ ID NO:2)

wherein A1 and A2 are amino acid residues. This tetra-peptide having hydrophobic or non-charged moieties (A and B) at the N and C terminals and which is an antioxidant by itself, is hydrolyzable *in vivo* to yield additional 14 antioxidant species, each having at least one cysteine residue, each of which is active in effecting antioxidation by virtue of the functional CH_2-SH-group of the cysteine residue thereof:

1. A-Cys (SEQ ID NO:3)
2. A-Cys-A1 (SEQ ID NO:4)

3. A-Cys-A1-A2 (SEQ ID NO:5)
4. A-Cys-A1-A2-Cys (SEQ ID NO:6)
5. Cys-A1-A2-Cys-B (SEQ ID NO:7)
6. A1-A2-Cys-B (SEQ ID NO:8)
7. A2-Cys-B (SEQ ID NO:9)
8. Cys-B (SEQ ID NO:10)
9. Cys-A1-A2-Cys (SEQ ID NO:11)
10. Cys-A1-A2 (SEQ ID NO:12)
11. Cys-A1 (SEQ ID NO:13)
12. Cys (SEQ ID NO:14)
13. A1-A2-Cys (SEQ ID NO:15)
14. A2-Cys (SEQ ID NO:16)

A specific example of an A-Cys-A1-A2-Cys-B (SEQ ID NO:2) tetrapeptide antioxidant compound is N-Acetyl Cysteine-Glycine-Proline-Cysteine-Amid (SEQ ID NO:1), which compound is designated in the Examples section that follows as CB and has the following chemical structure:

$$CH_3CO\text{-}NH\text{-}CH(CH_2SH)CO\text{-}NHCH_2CO\text{-}N(CH_2\text{-}CH_2\text{-}CH_2)\text{-}CO\text{-}NH\text{-}CH(CH_2SH)\text{-}CO\text{-}NH_2$$

Another compound which has the above listed properties and which is hydrolyzable within a cell so as to generate a plurality of antioxidant species acting in concert is for example the tripeptide having the general formula:

A-Cys-A1-Cys-B (SEQ ID NO:17)

This tripeptide can be hydrolyzed *in vivo* to yield additional 9 species, each having at least one cysteine residue which is active in effecting antioxidation by virtue of the functional CH_2-SH-group thereof:

1.	A-Cys	(SEQ ID NO:3)
2.	A-Cys-A1	(SEQ ID NO:4)
3.	A-Cys-A1-Cys	(SEQ ID NO:18)
4.	Cys-A1-Cys-B	(SEQ ID NO:19)
5.	Cys-A1-Cys	(SEQ ID NO:20)
6.	A1-Cys	(SEQ ID NO:21)
7.	Cys-A1	(SEQ ID NO:13)
8.	Cys-B	(SEQ ID NO:10)
9.	Cys	(SEQ ID NO:14)

Additional examples of tripeptides in accordance with the teaching of the present invention which include a single cysteine residue are:

1.	A-A1-Cys-A2-B	(SEQ ID NO:22)
2.	A-Cys-A1-A2-B	(SEQ ID NO:23)
3.	A-A1-A2-Cys-B	(SEQ ID NO:24)

each of which is hydrolyzable into 7 additional antioxidant species, acting in concert.

It will be appreciated in this respect that living cells include a repertoire of peptidases capable of hydrolyzing a peptide bond formed between any pair of amino acid residues in a peptide. Some peptidases are more specific than others, they may have different abundancy and subcellular distribution, so as to result in some antioxidant species being more represented than others in a certain cellular environment.

To successfully protect biological systems from oxidants, the antioxidant must have a higher reactivity for the oxidant than the biologic molecule which it seeks to protect. To protect the desired biologic system from oxidation, it is also necessary for the antioxidant to partition itself adjacent to the molecule to be protected. As an example, a molecule to be

protected within the lipid bilayer of plasma, endosomal or nuclear membranes might be best protected by an antioxidant with, at least in part, a lipophilic structure, so that it is partitioned to or near the lipid portion of the membrane, adjacent to the molecule needing protection from oxidation.

The hydrophobic or non-charged moieties conjugated to the antioxidant peptides of the present invention can be of any type which will render the compound sufficiently hydrophobic or non-charged so as to penetrate into the cytoplasm via its membrane miscibility properties. The exact type will of course depend on the peptide itself, as some peptides are more hydrophobic or non-charged than others. For central nervous system and other applications the compound of the present invention should be designed sufficiently hydrophobic or non-charged so as to cross blood barriers, such as, blood brain barrier, blood retinal barrier and blood testis barrier.

In addition to peptidases, living cells are also characterized by a large repertoire of other hydrolases such as, but not limited to, esterases and amidases, which are effective in hydrolyzing the bonds between the hydrophobic or non-charged moieties A and/or B and the peptide in-between, so as to increase the repertoire of antioxidant species released inside the cell. This cleavage action has an additional effect. Removal of one or both of the hydrophobic or non-charged moieties results in decrease in the total hydrophobic or non-charged moiety of the antioxidant species thus generated and as a result, the formed species are advantageously trapped in the cells, so as to efficiently exert their antioxidant properties therein.

Thus, according to a preferred embodiment of the present invention cleavage of the first bond and/or the second bond which connect between the hydrophobic or non-charged moieties A and/or B by a cellular hydrolase results in loosing the membrane miscibility, therefore the

antioxidant species are trapped within the cell so as to exert their antioxidant activity.

Each of the first and the second hydrophobic or non-charged moieties can independently be, for example, alkyl, aryl, alkene, arene or cholesteril having a backbone of 1-50 carbon atoms.

As used herein in the specification and in the claims section that follows, the term "alkyl" refers to a saturated aliphatic hydrocarbon group having a linear or branched backbone. Preferably, the alkyl has 1 to 20 carbon atoms in its backbone. Whenever a numerical range, e.g., "1-20", is stated herein, it means that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms. More preferably, the alkyl is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, it is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, cycloalkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioa, cyano, halo, carbonyl, thiocarbonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, nitro, sulfonamido, trihalomethanesulfonamido, silyl, guanyl, guanidino, ureido, amino or $NR_{10}R_{11}$, wherein R_{10} and R_{11} are each independently hydrogen, alkyl, cycloalkyl, aryl, carbonyl, sulfonyl, trihalomethysulfonyl and, combined, a five- or six-member heteroalicyclic ring.

A "cycloalkyl" group refers to an all-carbon monocyclic or fused ring (i.e., rings which share an adjacent pair of carbon atoms) group wherein, one of more of the rings does not have a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, cycloheptane, cycloheptatriene, and adamantane. A cycloalkyl group may be substituted or unsubstituted. When substituted, the

substituent group can be, for example, alkyl, aryl, heteroaryl, heteroalicyclic, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, halo, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, O-carbamyl, N-carbamyl, C-amido, N-amido, nitro, amino and $NR_{10}R_{11}$ as defined above.

An "alkenyl" group refers to an alkyl group which consists of at least two carbon atoms and at least one carbon-carbon triple bond.

An "aryl" group refers to an all-carbon monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, halo, trihalomethyl, alkyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thiocarbonyl, C-carboxy, O-carboxy, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, sulfinyl, sulfonyl, amino and $NR_{10}R_{11}$ as defined above.

A "heteroaryl" group refers to a monocyclic or fused ring (i.e., rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms, such as, for example, nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups include pyrrole, furane, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline and purine. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, cycloalkyl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thiocarbonyl, sulfonamido, C-carboxy, O-carboxy, sulfinyl, sulfonyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, amino or $NR_{10}R_{11}$ as defined above.

A "heteroalicyclic" group refers to a monocyclic or fused ring group having in the ring(s) one or more atoms such as nitrogen, oxygen and sulfur.

The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic may be substituted or unsubstituted. When substituted, the substituted group can be, for example, alkyl, cycloalkyl, aryl, heteroaryl, halo, trihalomethyl, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, carbonyl, thiocarbonyl, C-carboxy, O-carboxy, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, sulfinyl, sulfonyl, C-amido, N-amido, amino and $NR_{10}R_{11}$ as defined above.

According to a presently most preferred embodiment of the present invention, each of the hydrophobic or non-charged moieties identified herein by A and B; is independently N-acetyl, tert butyl, iso propyl, n-butyl n-pentyl, amide or ester.

A compound according to the present invention can be administered to an organism, such as a human being or any other mammal, *per se*, or in a pharmaceutical composition where it is mixed with suitable carriers or excipients.

Thus, according to another aspect of the present invention there is provided a pharmaceutical composition for preventing or reducing oxidative stress, the composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, an antioxidant compound as described hereinabove.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the compounds described herein, or physiologically acceptable salts or prodrugs thereof, with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium

carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Pharmaceutical compositions may also include one or more additional active ingredients, such as, but not limited to, anti inflammatory agents, antimicrobial agents, anesthetics and the like in addition to the antioxidant compounds.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain

tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a

suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The compounds of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

The pharmaceutical compositions herein described may also comprise suitable solid of gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin and polymers such as polyethylene glycols.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are

contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of antioxidant preparation effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

Toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the IC_{50} and the LD_{50} (lethal dose causing death in 50 % of the tested animals) for a subject compound. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Depending on the severity and responsiveness of the condition to be treated, dosing can also be a single administration of a slow release composition, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

The present invention can be used to treat any one of a plurality of diseases, disorders or conditions associated with the formation of oxidative stress.

As used herein, the term “treat” include substantially inhibiting, slowing or reversing the progression of a disease, disorder or condition,

substantially ameliorating clinical symptoms of a disease disorder or condition, or substantially preventing the appearance of clinical symptoms of a disease, disorder or condition.

The compounds according to the present invention can be used to treat central nervous system neurodegenerative disorders such as, but not limited to, Parkinson's disease, Alzheimer's disease, Creutzfeldt-Jakob's disease, cerebral ischemia, Multiple Sclerosis, basal ganglia degenerative disease, motoneuron diseases, Scrapie, spongyform encephalopathy and loss or impaired memory, peripheral tissue disorders such as, but not limited to, acute respiratory distress syndrome, amyotrophic lateral sclerosis, atherosclerotic cardiovascular disease, multiple organ dysfunction, complication resulting from inflammatory processes, AIDS, cancer and/or aging., all of which were previously shown to be associated with the formation and/or overproduction of oxidants and habits resulting in oxidative stress, such as, but not limited to, smoking, sun tanning, cancer treatment, radiation cocaine consumption and morphine consumption.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

EXAMPLE 1

Synthesis of N-Acetyl Cysteine-Glycine-Proline-Cysteine-Amid

The synthesis of N-Acetyl Cysteine-Glycine-Proline-Cysteine-Amid (CB, SEQ ID NO:1) having the chemical structure of:

CH_3CO-NH-CH(CH_2SH)CO-NHCH_2CO-N(CH_2-CH_2-CH_2)CH-CO-NH-CH(CH_2SH)-CO-NH_2

(molecular weight of 406) is described herein.

Synthesis: CB was prepared by solid phase synthesis of peptides according to published protocols. The synthesis was carried out according to Fastmoc 0.25 mmol modules in a peptide synthesizer Model 433A (Applied Biosystems) according to the User's manual.

In particular, 9-fluorenylmethoxycarbonyl (Fmoc) amino acid (1 mmol) was dissolved and activated in the cartridge in a mixture of 3.0 g of 0.45 M 2-(1H-benzoltriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU/HOBt) in DMF, 2 M Diisopropyethylamine (DIEA) and 0.8 ml N-methyl-pyrrolidone (NMP). De-protection was carried out in 22 % piperidine solution in NMP. All steps were carried out under nitrogen.

De-protection: The resin was de-protected as follows: Fmoc-Benzhydrylamine resin (368 mg; 0.25 mmol) was stirred in N-methyl pyrrolidone (7 ml). De-protection was carried out by washing the resin with 22 % piperidine/NMP solution for 2 minutes. The solvents were removed and the resin was subjected to a second treatment with 22 % piperidine/NMP for 7.6 minutes. Then, the resin was washed 6 times with DCM, followed by 4 washes in NMP.

Step 1: Fmoc-trityl cysteine (0.454g) was reacted for 6 min in NMP (2 g) together with 0.9 mmol of 0.45M HPTU/HOBt in DMF (2 g). De-protection was carried out as outlined above.

Step 2: Fmoc-proline (0.478 g) was reacted for 6 min in NMP (2 g) together with 0.9 mmol of 0.45 M HPTU/HOBt in DMF (2 g). De-protection was carried out as outlined above.

Step 3: Fmoc-glycine (0.493 mg) was reacted for 6 min in NMP (2 g) together with 0.9 mmol of 0.45 HPTU/HOBt in DMF (2 g). De-protection was carried out as outlined above.

Step 4: Fmoc-trityl cysteine (0.454 g) was reacted for 6 min in NMP (2 g) together with 0.9 mmol of 0.4 5M HPTU/HOBt in DMF (2 g). De-protection was carried out as outlined above.

Step 5: Acetic anhydride (0.534 g) was reacted 6 min in NMP (2 g) together with 0.9 mmol of 0.45M HPTU/HOBt in DMF (2 g).

Step 6: The resin was mixed using a vortex with 95 % TFA/2.5 % DDW/2.5% triisopropyl silane for 10 min at 40 °C and 2 hours at room temperature. The mixed resin was filtered and the resulting peptide was precipitated with cold ether. The precipitate was washed 4 times with cold ether, next 10 % acetic acid was added followed by lyophilization.

The yield of the above synthesis was 80 mg of the CB molecule.

Analysis: The product of the above synthesis was analyzed by HPLC. The HPLC profile of the purified CB compound is presented in Figure 1. Mass spectra of CB is 419.9. Amino acid data: is Gly - retention time of 13.35 min; 401.023 nmol/ml; Pro - retention time of 20.83 min; 402.56 nmole/ml; Cys - degraded.

EXAMPLE 2

Inhibition of JNK (c-Jun NH_2-terminal kinase) and p38 enzymes

In order to show the efficacy of CB against a stimulant that activates oxidative stress, an inhibition assay of both JNK (c-Jun NH2-terminal kinase) and p38 enzymes in tissue culture was performed.

NIH3T3 cells overexpressing EGF receptor (DHER14 cells, Dull et al., 1989) were exposed to cisplatin (CDDP, 30 μM) which activates specific enzymes involved in apoptosis including JNK and p38.

As shown in Figure 2, JNK or p38 were detected by specific antibodies essentially as described in Saitoh et al., 1998. In the presence of increasing concentrations of CB, a dramatic reduction in the phosphrylated form of either p38 or JNK enzymes was obtained. In the presence of 20 μM CB, phosphorylated p38 and JNK enzymes were not detected at all. Two

known antioxidants were used as positive controls, NOXi (at 300 and 1000 μM) and N-acetylcysteine (NAC) (at 1000 μM). The efficacy of CB at 20 μM was similar to that obtained by the addition of 1 mM of N-acetyl cysteine.

EXAMPLE 3

Inhibition of ROS production

The concentration of reactive oxygen species (ROS) in DHER14 cells following administration of antioxidants was determined using the ROS sensitive fluorescent dye DHDCF (fluoresceine derivative). As shown in Figure 3, reduction of ROS below normal levels was prominent in the presence of 20 μM of CB. Two known antioxidants were used as control, NOXi (at 1000 μM) and N-acetylcysteine (NAC) (at 1000 μM). The efficacy of CB in reducing ROS was about ~ 50 fold better then these two known antioxidants. Thus, at 20 μM CB was as efficient as 1000 μM NAC or 1000 μM NOXi.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

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SEQUENCE LISTING

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WHAT IS CLAIMED IS:

1. An antioxidant compound comprising:

(a) a peptide including at least three amino acid residues of which at least one being a cysteine residue having a readily oxidizable sulfhydryl group for effecting antioxidation; and at least one peptide bond cleavable by at least one intracellular peptidase; and

(b) a first hydrophobic or non-charged moiety being attached to an amino terminal of said peptide via a first bond and a second hydrophobic or non-charged moiety being attached to a carboxy terminal of said peptide via a second bond, said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety are selected so as to provide the antioxidant compound with membrane miscibility properties for permitting the antioxidant compound to cross cellular membranes;

wherein cleavage of said at least one peptide bond by said at least one intracellular peptidase results in generation of at least one antioxidant species including said cysteine residue having said readily oxidizable sulfhydryl group and which is also active in effecting antioxidation, thereby providing for a plurality of different antioxidant species.

2. The antioxidant compound of claim 1 having a general formula of:

A---Y1---Cys---Y2---Cys---Y3---B

or

A---Y1---Cys---Y2---B

wherein, Cys is a cysteine residue, A is the first hydrophobic or non-charged moiety; B is the second hydrophobic or non-charged moiety; Y1, Y2 and Y3 are each individually one or more amino acid residues in the range of 0-30 residues, with the provision that Y1, Y2 and Y3 collectively provide for at least two amino acid residues in the peptide.

3. The antioxidant compound of claim 2, wherein A is selected from the group consisting of N-acetyl, tert butyl, iso propyl, n-butyl and n-pentyl.

4. The antioxidant compound of claim 2, wherein B is selected from the group consisting of amide and ester.

5. The antioxidant compound of claim 1, wherein cleavage of said first bond and/or said second bond by a cellular hydrolase results in loosing said membrane miscibility.

6. The antioxidant compound of claim 1, wherein cleavage of said first bond and/or said second bond by a cellular hydrolase results in formation of additional antioxidant species acting in synergy.

7. The antioxidant compound of claim 1, wherein said first bond and said second bond are each independently an ester or peptide bond.

8. The antioxidant compound of claim 1, wherein each of said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety is selected from the group consisting of alkyl, aryl, alkene, arene and cholesteril having a backbone of 2-50 carbon atoms.

9. The antioxidant compound of claim 1, wherein said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety are selected so as to enable the antioxidant compound to cross a blood barrier.

10. The antioxidant compound of claim 9, wherein said blood barrier is selected from the group consisting of a blood brain barrier, a blood retinal barrier and a blood testis barrier.

11. The antioxidant compound of claim 1, wherein said plurality of different antioxidant species act in synergy to exert antioxidation.

12. A pharmaceutical composition for preventing or reducing oxidative stress, the composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, an antioxidant compound, said antioxidant compound including:

(a) a peptide including at least three amino acid residues of which at least one being a cysteine residue having a readily oxidizable sulfhydryl group for effecting antioxidation; and at least one peptide bond cleavable by at least one intracellular peptidase; and

(b) a first hydrophobic or non-charged moiety being attached to an amino terminal of said peptide via a first bond and a second hydrophobic or non-charged moiety being attached to a carboxy terminal of said peptide via a second bond, said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety are selected so as to provide the antioxidant compound with membrane miscibility properties for permitting the antioxidant compound to cross cellular membranes;

wherein cleavage of said at least one peptide bond by said at least one intracellular peptidase results in generation of at least one antioxidant species including said Cysteine residue having said readily oxidizable sulfhydryl group and which is also active in effecting antioxidation, thereby providing for a plurality of different antioxidant species.

13. The pharmaceutical composition of claim 12, wherein said pharmaceutically acceptable carrier is selected from the group consisting of a thickener, a base, a buffer, a diluent, a surface active agent and a preservatives.

14. The pharmaceutical composition of claim 12 wherein said antioxidant compound having a general formula of:

A---Y1---Cys---Y2---Cys---Y3---B

or

A---Y1---Cys---Y2---B

wherein, Cys is a cysteine residue, A is the first hydrophobic or non-charged moiety; B is the second hydrophobic or non-charged moiety; Y1, Y2 and Y3 are each individually one or more amino acid residues in the range of 0-30 residues, with the provision that Y1, Y2 and Y3 collectively provide for at least two amino acid residues in the peptide.

15. The pharmaceutical composition of claim 14 wherein A is selected from the group consisting of N-acetyl, tert butyl, iso propyl, n-butyl and n-pentyl.

16. The pharmaceutical composition of claim 14, wherein B is selected from the group consisting of amide and ester.

17. The pharmaceutical composition of claim 12, wherein cleavage of said first bond and/or said second bond by a cellular hydrolase results in loosing said membrane miscibility.

18. The pharmaceutical composition of claim 12, wherein cleavage of said first bond and/or said second bond by a cellular hydrolase results in formation of additional antioxidant species acting in synergy.

19. The pharmaceutical composition of claim 12, wherein said first bond and said second bond are each independently an ester or peptide bond.

20. The pharmaceutical composition of claim 12, wherein each of said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety is selected from the group consisting of alkyl, aryl, alkene, arene and cholesteril having a backbone of 14-50 carbon atoms.

21. The pharmaceutical composition of claim 12, wherein said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety are selected so as to enable said antioxidant compound to cross a blood barrier.

22. The pharmaceutical composition of claim 21, wherein said blood barrier is selected from the group consisting of a blood brain barrier, a blood retinal barrier and a blood testis barrier.

23. The pharmaceutical composition of claim 12, wherein said plurality of different antioxidant species act in synergy to exert antioxidation.

24. A method of preventing or reducing oxidative stress, the method comprising the step of administering to a subject an effective amount of an antioxidant compound, said antioxidant compound including:

(a) a peptide including at least three amino acid residues of which at least one being a cysteine residue having a readily oxidizable sulfhydryl group for effecting antioxidation; and at least one peptide bond cleavable by at least one intracellular peptidase; and

(b) a first hydrophobic or non-charged moiety being attached to an amino terminal of said peptide via a first bond and a second hydrophobic or non-charged moiety being attached to a carboxy terminal of said peptide via a second bond, said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety are selected so as to provide the antioxidant compound with membrane miscibility properties for permitting the antioxidant compound to cross cellular membranes;

wherein cleavage of said at least one peptide bond by said at least one intracellular peptidase results in generation of at least one antioxidant species including said cysteine residue having said readily oxidizable sulfhydryl group and which is also active in effecting antioxidation, thereby providing for a plurality of different antioxidant species.

25. The method of claim 24, wherein said subject is a human being.

26. The method of claim 24, wherein said subject has a peripheral disorder characterized by formation of oxidative stress.

27. The method of claim 26, wherein said peripheral disorder characterized by formation of oxidative stress is selected from the group consisting of an acute respiratory distress syndrome, an amyotrophic lateral sclerosis, an atherosclerotic cardiovascular disease, multiple organ dysfunction, complication resulting from inflammatory processes, AIDS, cancer and aging.

28. The method of claim 24, wherein said subject has a neurodegenerative disorder characterized by oxidative stress.

29. The method of claim 28, wherein said neurodegenerative disorder characterized by oxidative stress. is selected from the group consisting of Parkinson's disease, Alzheimer's disease, Creutzfeldt-Jakob's disease, cerebral ischemia, Multiple Sclerosis, basal ganglia degenerative disease, motoneuron diseases, Scrapie, spongyform encephalopathy and loss or impaired memory.

30. The method of claim 24, wherein said subject has at least one habit resulting in oxidative stress.

31. The method of claim 30, wherein said habit is selected from the group consisting of smoking, sun tanning, cancer treatment, radiation cocaine consumption and morphine consumption.

32. The method of claim 24, wherein said administration is a peripheral administration.

33. The method of claim 32, wherein said peripheral administration is selected from the group consisting of topical

administration, oral administration, administration by inhalation, and parenteral administration.

34. The method of claim 24, wherein said compound having a general formula of:

A---Y1---Cys---Y2---Cys---Y3---B

or

A---Y1---Cys---Y2---B

wherein, Cys is a cysteine residue, A is the first hydrophobic or non-charged moiety; B is the second hydrophobic or non-charged moiety; Y1, Y2 and Y3 are each individually one or more amino acid residues in the range of 0-30 residues, with the provision that Y1, Y2 and Y3 collectively provide for at least two amino acid residues in the peptide.

35. The method of claim 34, wherein A is selected from the group consisting of N-acetyl, tert butyl, iso propyl, n-butyl and n-pentyl.

36. The method of claim 34, wherein B is selected from the group consisting of amide and ester.

37. The method of claim 24, wherein cleavage of said first bond and/or said second bond by a cellular hydrolase results in loosing said membrane miscibility.

38. The method of claim 24, wherein cleavage of said first bond and/or said second bond by a cellular hydrolase results in formation of additional antioxidant species acting in synergy.

39. The method of claim 24, wherein said first bond and said second bond are each independently an ester or peptide bond.

40. The method of claim 24, wherein each of said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety is selected from the group consisting of alkyl, aryl, alkene, arene and cholesteril having a backbone of 2-50 carbon atoms.

41. The method of claim 24, wherein said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety are selected so as to enable the antioxidant compound to cross a blood barrier.

42. The method of claim 41, wherein said blood barrier is selected from the group consisting of a blood brain barrier, a blood retinal barrier and a blood testis barrier.

43. The method of claim 24, wherein said plurality of different antioxidant species act in synergy to exert antioxidation.

Amendments to the claims have been filed as follows

1. An antioxidant compound comprising:

(a) a peptide including at least three amino acid residues of which at least two being cysteine residues each having a readily oxidizable sulfhydryl group for effecting antioxidation; and at least two peptide bonds each being cleavable by at least one intracellular peptidase; and

(b) a first hydrophobic or non-charged moiety being attached to an amino terminal of said peptide via a first bond and a second hydrophobic or non-charged moiety being attached to a carboxy terminal of said peptide via a second bond, said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety are selected so as to provide the antioxidant compound with membrane miscibility properties for permitting the antioxidant compound to cross cellular membranes;

wherein cleavage of said at least two peptide bonds by said at least one intracellular peptidase results in generation of several antioxidant species each including at least one of said cysteine residues having said readily oxidizable sulfhydryl group and which is also active in effecting antioxidation, thereby providing for a plurality of different antioxidant species acting in synergy in exerting antioxidation.

2. The antioxidant compound of claim 1 having a general formula of:

A---Y1---Cys---Y2---Cys---Y3---B

wherein, Cys is a cysteine residue, A is the first hydrophobic or non-charged moiety; B is the second hydrophobic or non-charged moiety; Y1, Y2 and Y3 are each individually one or more amino acid residues in the range of 0-30 residues, with the provision that Y1, Y2 and Y3 collectively provide for at least two amino acid residues in the peptide.

3. The antioxidant compound of claim 2, wherein A is selected from the group consisting of N-acetyl, tert butyl, iso propyl, n-butyl and n-pentyl.

4. The antioxidant compound of claim 2, wherein B is selected from the group consisting of amide and ester.

5. The antioxidant compound of claim 1, wherein cleavage of said first bond and/or said second bond by a cellular hydrolase results in loosing said membrane miscibility.

6. The antioxidant compound of claim 1, wherein cleavage of said first bond and/or said second bond by a cellular hydrolase results in formation of additional antioxidant species acting in synergy.

7. The antioxidant compound of claim 1, wherein said first bond and said second bond are each independently an ester or peptide bond.

8. The antioxidant compound of claim 1, wherein each of said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety is selected from the group consisting of alkyl, aryl, alkene, arene and cholesteril having a backbone of 2-50 carbon atoms.

9. The antioxidant compound of claim 1, wherein said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety are selected so as to enable the antioxidant compound to cross a blood barrier.

10. The antioxidant compound of claim 9, wherein said blood barrier is selected from the group consisting of a blood brain barrier, a blood retinal barrier and a blood testis barrier.

11. A pharmaceutical composition for preventing or reducing oxidative stress, the composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, an antioxidant compound, said antioxidant compound including:

(a) a peptide including at least three amino acid residues of which at least two being cysteine residues, each having a readily oxidizable sulfhydryl group for effecting antioxidation; and at least two peptide bonds each being cleavable by at least one intracellular peptidase; and

(b) a first hydrophobic or non-charged moiety being attached to an amino terminal of said peptide via a first bond and a second hydrophobic or non-charged moiety being attached to a carboxy terminal of said peptide via a second bond, said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety are selected so as to provide the antioxidant compound with membrane miscibility properties for permitting the antioxidant compound to cross cellular membranes;

wherein cleavage of said at least two peptide bonds by said at least one intracellular peptidase results in generation of a plurality of antioxidant species each including at least one of said cysteine residues having said readily oxidizable sulfhydryl group and which is also active in effecting antioxidation, thereby providing for a plurality of different antioxidant species acting in synergy in exerting antioxidation.

12. The pharmaceutical composition of claim 11, wherein said pharmaceutically acceptable carrier is selected from the group consisting of a thickener, a base, a buffer, a diluent, a surface active agent and a preservatives.

13. The pharmaceutical composition of claim 11 wherein said antioxidant compound having a general formula of:

A---Y1---Cys---Y2---Cys---Y3---B

wherein, Cys is a cysteine residue, A is the first hydrophobic or non-charged moiety; B is the second hydrophobic or non-charged moiety; Y1, Y2 and Y3 are each individually one or more amino acid residues in the range of 0-30 residues, with the provision that Y1, Y2 and Y3 collectively provide for at least two amino acid residues in the peptide.

14. The pharmaceutical composition of claim 13 wherein A is selected from the group consisting of N-acetyl, tert butyl, iso propyl, n-butyl and n-pentyl.

15. The pharmaceutical composition of claim 13, wherein B is selected from the group consisting of amide and ester.

16. The pharmaceutical composition of claim 11, wherein cleavage of said first bond and/or said second bond by a cellular hydrolase results in loosing said membrane miscibility.

17. The pharmaceutical composition of claim 11, wherein cleavage of said first bond and/or said second bond by a cellular hydrolase results in formation of additional antioxidant species acting in synergy.

18. The pharmaceutical composition of claim 11, wherein said first bond and said second bond are each independently an ester or peptide bond.

19. The pharmaceutical composition of claim 11, wherein each of said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety is selected from the group consisting of alkyl, aryl, alkene, arene and cholesteril having a backbone of 14-50 carbon atoms.

20. The pharmaceutical composition of claim 11, wherein said first hydrophobic or non-charged moiety and said second hydrophobic or non-charged moiety are selected so as to enable said antioxidant compound to cross a blood barrier.

21. The pharmaceutical composition of claim 20, wherein said blood barrier is selected from the group consisting of a blood brain barrier, a blood retinal barrier and a blood testis barrier.

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UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

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Other: ONLINE: WPI, JAPIO, EPODOC, TXTE, CAPLUS, EMBASE, BIOSIS, MEDLINE, SCISEARCH

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A	EP0354820A	(NIPPON CHEMIPHAR CO. LTD) See especially page 2 line 36 - page 3 line 18 and examples	
X	US5874468A	(ATLAS) See especially column 11 line 53- column 12 line 5, column 13 line 62- column 14 line 65, column 16 line 25-50 and examples	All
X	US5464825A	(ANDERSON) See especially column 3 line 65- column 4 line 33 and examples	All
A	*Biochem Mol Biol Int*; Vol 33 (6), pp 1041-1048 (1994). Ueda *et al*. See especially Results and Discussion		
A	*Circ Res*; Vol 74 (5), pp 806-816 (1994). Nakamura *et al*. See especially Results and Discussion		

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
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